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Research Article

The Research and Application of Systematic Technologies for Reducing Tobacco-Specific N-Nitrosamines in Tobacco and Cigarette Smoke

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Abstract

The systematic technologies for reducing cancerogenic Tobacco-Specific N-nitrosamines (TSNAs) in both agricultural and industrial area were developed and applied. The TSNA levels for the upper and middle leaves of burley tobacco could be reduced 23.0% and 23.9%, respectively, by foliage spray of purslane extract during tobacco cultivation. Through breed improvement of Maryland tobacco Wufeng#1to reduce nicotine conversion rate, TSNA levels for the upper and middle leaves of Maryland tobacco could be reduced 66.3% and 70.1%, respectively. By spraying 4.5% (W/W) of purslane extract and 0.4% (W/W) nanometer silica dispersion liquid during threshing and redrying, the TSNA level of tobacco could be reduced 36.0% and 20.5%, respectively, compared with that of the control. The level of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in the cigarette smoke could be selectively reduced 34.6% when 5% (W/W) of cytochrome P450 recombinase was added to processed burley tobacco in processing. Using 10% of reconstituted tobacco processed with 10% (W/W) of nanometer silica and 4% of cytochrome P450 recombinase could result in 20.0% decrease of TSNAs in cigarette smoke. The TSNA level in cigarette smoke could be reduced 24.1% by using complex cigarette filter containing 0.6 mg of modified nanometer silica and 16.8 mg of macroporous silica gel instead of cellulose acetate filter with the similar parameters. All the above TSNA reduction technologies combined and applied in cigarette manufacture could approach the selective reduction of 58% of TSNAs in cigarette smoke and the 45% decrease of cigarette hazard index.

Keywords: Tobacco-specific nitrosamines; Reduction; Systematic technologies; Research; Application

Abbreviations

TSNAs: Tobacco-Specific N-Nitrosamines; NNK: (4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone); NNN: N'-nitrosonornicotine; NAT: N'-nitrosoanatabine; NAB: N'-nitrosoanabasine; DRIFTS: Diffuse Reflectance Infrared Fourier Transform Spectroscopy; OPB: 4-oxo-4-(3-pyridyl) Butanal; HPB: 4-hydroxy-1-(3-pyridyl) -1-butanone.

Introduction

As a type of carcinogenic N-nitroso compound, Tobacco Specific N-nitrosamines (TSNAs) are products of the nitrozation reaction between tobacco alkaloids and nitrite [1]. There are four main types of TSNAs: NNK, (4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone), NNN (N'-nitrosonornicotine), NAT (N'-nitrosoanatabine), and NAB (N'-nitrosoanabasine). NNK and NNN are potential carcinogens for rodents and are classified as carcinogens on Cancer Group I by International Agency for Research [2-4]. TSNAs are found in tobacco leaves and cigarette smoke, but are present in very small (almost negligible) amounts in green tobacco leaves. The formation and accumulation of TSNAs mainly occurs during curing and storage [5-6]. Some TSNAs in cigarette smoke are transferred directly from tobacco, while others are formed and transmitted during smoking [7]. Comprehensive and systematic studies on technologies reducing TSNA levels for tobacco and cigarette smoke are of important significance for reducing the risk of smoking.

Multiple TSNA-reducing technologies are currently available. Some lower TSNA release was achieved by using longer filter tip, filter tip ventilation, tipping paper ventilation and cigarette paper ventilation or using cigarette papers with faster static burn rates [8-10]. While these methods do reduce TSNA release in some extent, there are significant draw backs; they alter the sensory quality of cigarettes due to the reduction of aromatic compounds in cigarette smoke [11-12]. Furthermore, there is no selectivity in the reduction of TSNAs. Some technologies are still under laboratory development and the new materials involved are either expensive or require complex preparatory techniques [13-15], which hampers industrial application. Moreover, the lack of variety for the current TSNA reduction techniques is an impediment to the development of a full technological series that lends itself easily to comprehensive and systematic applications.

From the perspective of tobacco agriculture and industry in which the reduction of TSNA levels can be achieved, this article provided a systematic series of applicable technologies for reducing TSNAs in tobacco and cigarette smoke. We examined tobacco cultivation,

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threshing and redrying, storage of tobacco, and cigarette processing to provide useful data and technical references to researchers studying technologies that aim to reduce the risk posed by smoking.

Materials and Methods

Experimental material

Burley tobacco -Eyan#1, TN90LC and TN86, Maryland tobacco -Wufeng#1, Wufeng#1 LC, flue-cured tobacco Yun 87 and purslane extract were obtained from Hubei Tobacco Research Institute. Nanometer silica was bought from Tai Hong Sheng Da New Material Co., Ltd., Tangshan, China). Cytochrome P450 recombinase was obtained from Beijing Ming Bo FeiScietific and Technological Company, Ltd, Beijing, China. Macroporous silica gel was bought from Anhui Liang ChenGui Yuan New Material Co., Ltd., Luan, China).NNN, NAT, NAB, NNK, NNN-d_4, NAT-d_4, NAB-d_4, and NNK-d_4 (purity of all >98%) were obtained from TRC Ltd, Canada. Glycol was bought from Guangzhou Xian Yan chemical company, Ltd, Guangzhou, China.

Experimental methods

TSNA reduction during tobacco agriculture processes: Maryland tobacco Wufeng#1 was used as research object. Screening was conducted on the nicotine conversion rate of this variety and non-transformants were cultivated for breed improvement. The nicotine conversion rate of tobacco leaves [16] and TSNA contents were measured [17] to compare changes in both parameters.

Purslane extract was made by filtrating fresh purslane that was mashed and ground in clean water at a purslane to water ratio of 1:1. On the day of tobacco topping, the extract was sprayed evenly on the foliage surface of burley tobacco Eyan#1 at 900L/ha. Tobacco leaves sprayed with water were employed as control. TSNA levels were measured after leaves had ripened and been collected and cured.

TSNA reduction during threshing and redrying: Burley tobacco Eyan#1 was used as research object in the experiment. Tobacco leaves were topped and de-stemmed. Before the tobacco leaves were put in the redrying machine, jute lint removal had been done. Purslane extracts accounting for 4.5% of the weight of the tobacco leaves and at a concentration of 12.5%, 25% and 50% was mixed to form a liquid suspension. The preparation was atomized by air compression and evenly automatically sprayed onto tobacco leaves. After redrying, the leaves were compared in terms of TSNA level differences with the control that were sprayed with water.

Flue-cured tobacco Yun 87 was used in the experiment. Nanometer silica was mixed with water and glycol to make dispersion liquid (10:88:2, W/W). The preparation was atomized by air compression and evenly automatically sprayed onto tobacco leaves (4%, W/W). Then the processed tobacco leaves were put into redrying machine for redrying. Nanometer silica-free tobacco leaves were used as the control. TSNA levels of both groups were measured after one year of natural storage under identical circumstances. In-situ infrared spectroscopic scanning was also conducted on the nanometer silica taken from nanometer silica processed tobacco and ordinary nanometer silica by Bruker Vertex 70 using a Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS).

TSNA formation inhibition during tobacco storage: Burley tobacco Eyan#1 and Maryland tobacco Wufeng#1 were used in the experiment. After threshing and redrying, tobacco leaves were stored for 2 years at a temperature of 20°C. The difference in TSNA levels was compared with the control stored at ambient temperature (the storage temperature changed along with the ambient temperature).

Burley tobacco TN86 was used in the experiment. After threshing and redrying, tobacco leaves were packaged in cartons (ordinary packaging), plastic bags, and vacuum packaging, and then stored naturally under identical circumstances for one year. Leaves packaged in cartons were used as the control. The levels of TSNAs and neutral aroma constituents [18] of the tobacco with different packaging were measured.

TSNA reduction during cigarette manufacturing: Cytochrome P450 recombinase (5% of the weight of burley tobacco leaves) was mixed into the flavor preparations. During the flavor spraying procedure, a foliage spray of the cytochrome P450 recombinase mixture was automatically applied to burley tobacco leaves evenly by the atomization of compressed air. After 2 hours of storage, processing was conducted in accordance with the leaf group formula for cigarette manufacturing. Cigarettes made in the absence of cytochrome P450 recombinase were the control. The amount of TSNAs in the mainstream smoke of the experimental cigarettes and the control was determined [19].

During the processing of reconstituted tobacco leaves, cytochrome P450 recombinase was added to the concentrate prepared after raw tobacco materials had been extracted. The volume added accounted for 4% (W/W) of the concentrate. After 24 hours, the concentrate in which NNK had been fully degraded by cytochrome P450 recombinase was formed into a coating liquid and 10% (W/W) nanometer silica was added. The mixture was applied to tobacco leaves by dip-coating to produce functionally reconstituted tobacco for reducing TSNAs. Cigarettes were manufactured with the tobacco shreds containing 10% of the functional reconstituted tobacco. Cigarettes made with the tobacco shreds containing 10% of ordinary reconstituted tobacco instead of the functional reconstituted tobacco were used as the control. The levels of TSNAs in the mainstream smoke of the experimental cigarettes and the control were measured.

During the manufacture of cigarette filters, modified nanometer silica was evenly distributed in glycerin triacetate at 8%W/W). The preparation was then evenly sprayed onto acetate tow at an amount of 8%W/W). At the same time, macroporous silica gel granules were added to acetate tow at 1.12mg/mm. The feeding filter rods were combined with ordinary cellulose acetate filter rods at a ratio of 15:10 for the manufacture of complex filter rods. The complex filter rods were used in the manufacture of cigarettes. Cigarettes using ordinary filters with the similar parameters and the same tobacco shreds were employed as the control. The amount of TSNAs in the mainstream smoke of the experimental cigarettes and the control was determined.

Application of the systematic TSNA-reducing technologies: From 2011-2017, the above TSNA-reducing technologies were gradually applied in new cigarette products development and commercial cigarettes maintenance. For example, Zhongnanhai (ZNH) is a key cigarette brand that the systematic TSNA-reducing technologies were applied. The level of TSNAs in the cigarette smoke and cigarette hazard index (H index = [Y_{CO}/14.2 + Y_{HCN}/146.3 +

Table 1: Nicotine conversion rate and TSNA levels for Wufeng #1 and Wufeng #1 LC.

Variety	Nicotine conversion rate /%	Leaf area	NNN/(ng.g-1)	NAT/(ng.g-1)	NAB/(ng.g-1)	NNK/(ng.g-1)	TSNAs/(ng.g-1)
Wufeng #1	16.50	Upper	12653.2	1404.3	113.7	88.9	14260.1
		Middle	11545.8	1159.4	99.8	62.3	12867.2
Wufeng #1 LC	2.42	Upper	3310.7	1291.5	101.9	105.0	4809.0
		Middle	2645.1	1016.6	114.8	70.4	3846.9

Table 2: Effects of purslane extract on TSNA levels for burley tobacco.

Types	Leaf area	Treatment	NNN /(ng.g-1)	NAT/(ng.g-1)	NAB/(ng.g-1)	NNK/(ng.g-1)	TSNAs/(ng.g-1)
Burley tobacco	Upper	Control	37421.5	9756.3	368.7	585.1	48131.6
		Purslane extract	29471.5	6920.4	253.3	407.7	37052.9
	Middle	Control	45234.2	11462.2	457.9	640.1	57794.4
		Purslane extract	34715.6	8475.1	384.2	431.5	44006.4

Table 3: Effects of purslane extract spraying on TSNA levels for burley tobacco during tobacco threshing and redrying.

Types	Treatment	NNN/(ng.g-1)	NAT/(ng.g-1)	NAB/(ng.g-1)	NNK/(ng.g-1)	TSNAs/(ng.g-1)
Burley tobacco	Control	12408.8	3984.3	188.7	569.2	17151.0
	12.5% purslane extract	10627.5	3324.7	158.6	479.4	14590.2
	25% purslane extract	8259.9	2264.2	131.2	323.8	10979.1
	50% purslane extract	8874.5	2501.4	140.0	351.5	11867.4

Table 4: Effects of adding nanometer silica on TSNA levels for tobacco during storage.

Treatment	NNN/(ng.g-1)	NAT/(ng.g-1)	NAB/(ng.g-1)	NNK/(ng.g-1)	TSNAs/(ng.g-1)
Control	76.4	108.7	3.6	30.9	219.6
Tobacco processed with nanometer silica	60.9	87.4	2.8	23.5	174.6

$Y_{NNK}/5.5 + Y_{NH3}/8.1 + Y_{B[a]P}/10.9 + Y_{PHE}/17.4 + Y_{CRO}/18.6] \times 10/7$, where Y is the yield of a hazardous component in mainstream cigarette smoke) for product ZNH were checked annually.

All the above TSNA-reducing technologies were applied in the development of product B, which was a newly developed product with a designed tar level of 8 mg/cig. Cigarettes manufactured without using the above TSNA-reducing technologies, but using the ordinary cigarette materials of similar parameters and same manufacturing parameters were used as the control.

Results and Discussion

TSNAs reduction in tobacco agriculture field

TSNA reduction by breed improvement: Wufeng#1 LC with low nicotine conversion rate was obtained by culturing non-transformed tobacco plant through nicotine conversion rate screening. Compared to Wufeng#1, the nicotine conversion rate for Wufeng#1 LC decreased by 85.33%, from 16.50% to 2.42%. The NNN level and the TSNA level for the upper leaves decreased by 73.84% and 66.28%, respectively, compared with the control. The NNN level and the TSNA level for the middle leaves decreased by 77.09% and 70.10%, respectively, compared with the control. The results are shown in Table 1.

TSNA reduction by spraying purslane extract: The extract of purslane is rich in flavonoids, which can react with nitrites to inhibit TSNA formation. About 7% of purslane extract are flavonoids. So, the extract of purslane is used to remove nitrite from tobacco leave to inhibit TSNA formation. The application of purslane extract during the period of tobacco leaf topping was selected to reduce the levels of TSNAs. As shown in table 2, the application of extract of purslane could decrease the NNK level by 30.32% and TSNA level by 23.02% for upper leaves of burley tobacco. The NNK level and TSNAs level for the middle leaves of burley tobacco decreased by 32.59% and by 23.86%, respectively, compared with the control.

TSNA reduction during threshing and redrying

TSNA reduction by spraying purslane extract: The procedures for reducing TSNAs by spraying purslane extract during tobacco threshing and redrying were listed below (Figure 1). The best results were obtained by spraying the purslane extract with a concentration of 25%, which reduced the NNK level of burley tobacco by 43.11% and TSNA level by 35.99%, respectively (Table 3).

Inhibition of TSNA formation by absorption of nitric oxides generated during storage by nanometer silica: Through exploring the formation mechanism of TSNAs in tobacco leaves during the storage, it is clear that the formation of TSNAs is mainly related to the nitrogen oxides, which is generated from nitrates and nitrites [20]. Sonanometer silica which has large surface area and strong nitrogen oxide adsorption capacity was employed to absorb nitrogen oxides generated from tobacco to inhibit TSNA formation. After one year of natural storage, the NNK and TSNA level of the tobacco leaves processed with nanometer silica decreased by 23.95% and 20.49%, respectively (Table 4).

The tobacco processed with nanometer silica and the control tobacco was scanned by in situ infrared spectroscopy during tobacco storage. It is confirmed that the nanometer silica actually absorbed nitrogen oxides released from tobacco leaves according to the

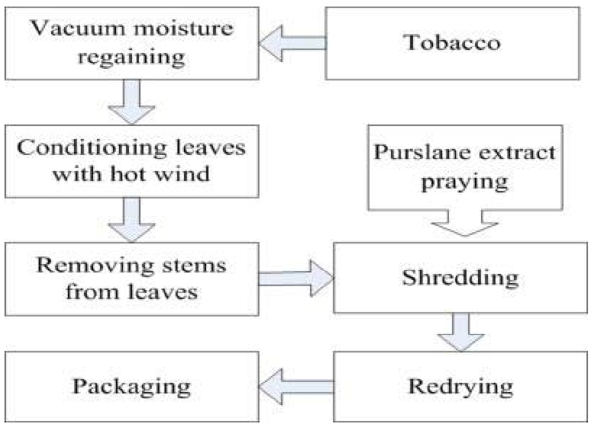

Figure 1: Procedures for reducing TSNAs by spraying purslane extract during tobacco threshing and redrying.

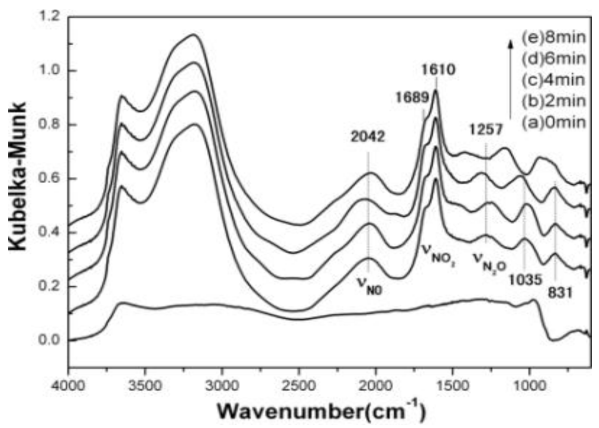

Figure 2: Infrared spectra of the nanometer silica taken from nanometer silica processed tobacco and ordinary nanometer silica.

infrared characteristic absorption and wave number characterization (Figure 2).

TSNA reduction during tobacco leaf storage

Inhibition of TSNA formation by controlling storage temperature: Previous studies have investigated the influence of temperature and moisture at storage on TSNA levels for tobacco [21-22]. The TSNA levels for tobacco increased along with the increase of temperature above 30°C, however, with the decrease of moisture (Figure 3).

Compared with burley and Maryland tobacco which stored at ambient temperature, that tobacco stored at 20°C for 2 years had lower levels of TSNAs. As shown in Table 5, NNK and TSNA level of burley tobacco reduced 47.00% and 49.47%, respectively, compared to that of the control. NNK and TSNA level of Maryland tobacco reduced 47.79% and 49.64%, respectively, compared to that of the control.

Inhibition of TSNA formation during storage using vacuum packaging: The TSNA levels of tobacco were influenced differently by varied packaging. After one year of ambient temperature storage, ordinary packaging and plastic bag packaging had similar NNK and TSNA level. However, the NNK and TSNA level of tobacco using

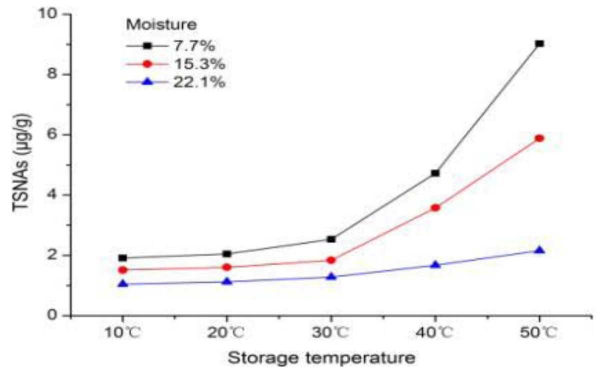

Figure 3: Effect of temperature and moisture on TSNA levels for tobacco leaves during storage.

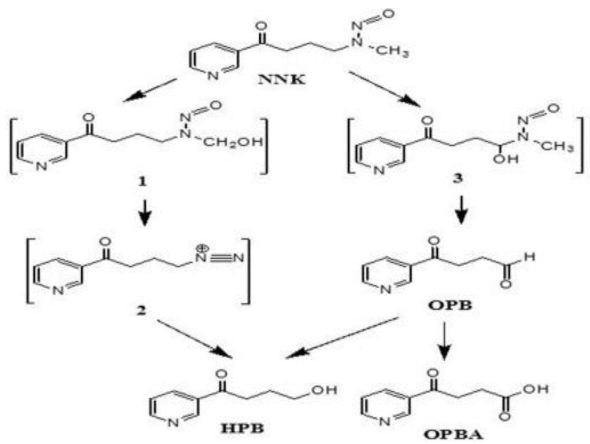

Figure 4: *In vitro* NNK degradation pathways under the action of cytochrome P450 recombinase.

vacuum packaging could be 37.90% and 45.98% lower than using ordinary packaging (Table 6). Therefore, vacuum packaging could effectively inhibit suppress TSNA formation during storage.

The levels of neutral aroma constituents for burley tobacco with the three packaging showed significant differences after 1 year of storage. The total level of neutral aroma constituents for tobacco with vacuum packaging was the highest (636.225μg/g).The total level of neutral aroma constituents for tobacco with plastic bag was the second highest (564.453μg/g). The lowest level of neutral aroma constituents (494.570μg/g) was for the tobacco packaged with carton (Table 7).

TSNA reduction during cigarette manufacture

TSNAs reduction using cytochrome P450 recombinase during tobacco processing: Through in-depth study of 869 tobacco endophyte and isolation, screening and cultivation, excellent strains which can reduce TSNAs were obtained. At the same time, genomic DNA identification of excellent strains was carried out. Cytochrome oxidase P450 (CYP2A1, CYP2A6) that can selectively degrade NNK *in vivo* was found. So, cytochrome P450 recombinase was employed to selectively reduce NNK and the degradation mechanism of NNK *in vitro* by cytochrome P450 recombinase was also studied [23-24]. A new pathway for NNK degradation *in vitro* was found for the first

Table 5: Effect of low temperature storage on TSNA levels of burley tobacco and Maryland tobacco.

Types	Treatment	NNN/(ng.g-1)	NAT/(ng.g-1)	NAB/(ng.g-1)	NNK/(ng.g-1)	TSNAs/(ng.g-1)
Burley tobacco	normal temperature storage	15263.1	7562.1	214.8	567.2	23607.2
	20°C storage	7421.1	4078.7	128.9	300.6	11929.3
Maryland tobacco	normal temperature storage	12345.8	4202.6	134.9	402.6	17085.9
	20°C storage	5897.1	2415.7	81.2	210.2	8604.2

Table 6: TSNA levels for burley tobacco with different packaging during storage.

Packaging methods	NNN/(ng.g-1)	NAT/(ng.g-1)	NAB/(ng.g-1)	NNK/(ng.g-1)	TSNAs/(ng.g-1)
Carton	6301.6	5984.0	67.4	72.3	12425.3
Plastic bag	5781.5	6386.5	63.8	68.8	12300.6
Vacuum packaging	3102.3	3528.9	36.3	44.9	6712.4

Table 7: Levels of neutral aroma constituents for burley tobacco with different packaging during storage.

Types	Neutral aroma constituents	Content/(μg.g-1)		
		Carton	Plastic bag	Vacuum packaging
Degradation products of carotenoid	Dihydroactinidiolide	3.8	3.3	4.4
	3-Hydroxy-β-damascone	2.7	3.1	6.1
	Isophorone oxide	0.3	0.3	0.5
	Isophorone	0.4	0.4	0.7
	Megastigmatrienone 1	2.4	2.7	5.0
	Megastigmatrienone 2	11.4	13.3	24.6
	Megastigmatrienone 3	1.4	2.3	2.0
	Megastigmatrienone 4	13.1	14.6	25.9
	Damascone	13.4	13.5	13.1
	Methylheptenone	1.2	1.2	1.2
	6-Methyl-5-Hepten-2-ol	0.2	0.2	0.3
	Geranyl acetone	2.0	2.1	2.9
	Farnesyl acetone	12.0	11.8	13.2
	Linalool	1.7	1.5	1.4
	Solavetivone	0.2	0.6	0.5
	β-Damascone	10.8	10.2	10.9
	Guaiacol	1.0	0.9	1.0
	Total	78.1	82.0	113.7
Cembratriendiol group	Solanone	28.4	27.1	32.1
Phenylalanine group	Benzaldehyde	1.7	2.1	2.5
	Phenylcarbinol	4.1	3.0	3.3
	Phenylacetaldehyde	17.5	19.7	19.2
	Phenethyl alcohol	6.1	5.2	6.4
	Total	29.4	30.0	31.4
Browning reaction group	Furfural	12.6	8.9	8.7
	Furfuralcohol	3.6	1.8	3.1
	2-Acetylfuran	0.2	0.2	0.2
	5-Methyl furfural	6.3	5.0	5.4
	3,4-Dimethyl-2,5-furandione	1.0	1.0	0.9
	2,6-Nonadienal	1.6	0.7	1.0
	Safranal	0.1	0.1	0.1
	β-Cyclocitral	0.3	0.3	0.2
	Total	25.5	17.8	19.8
Neophytadiene	Neophytadiene	333.1	407.6	439.2
Total		494.6	564.5	636.2

time. Under the action of cytochrome P450 recombinase, NNK was methylene hydroxylated to form intermediate 3, from which CH_4-N_2O was removed and 4-Oxo-4-(3-Pyridyl) Butanal (OPB) was formed. Then OPB was enzymatically hydrolyzed to form 4-Hydroxy-1-(3-Pyridyl) -1-Butanone (HPB). As shown in Figure 4.

The TSNA levels for the mainstream smoke of experimental cigarettes and the control were shown in Table 8. Compared with the control cigarettes, the level of TSNAs and NNK in the mainstream smoke of experimental cigarettes with cytochrome P450 recombinase processed tobacco shreds was selectively decreased by 10.14% and 34.64%, respectively.

TSNA reduction using functional reconstituted tobacco leaves produced with cytochrome P450 recombinase andnanometer silica: The method and proportion of adding nanometer silica and cytochrome P450 series recombinase preparations in the processing of reconstituted tobacco leaves were studied. It was clear that cytochrome P450 series recombinase preparations were added to the concentrate prepared from the raw materials of tobacco leaves. The proportion of adding cytochrome P450 recombinase preparations accounted for 4% (W/W) of the total concentrate. The treated concentrate by cytochrome P450 recombinase was to prepare coating solution. Then, 10% (W/W) nanometer silica were mixed into coating solution and added to the reconstituted tobacco leaves by coating procedure. The reconstituted tobacco leaves with the function of reducing TSNAs were produced. Compared with the control cigarettes with the common reconstituted tobacco leaves, the release of TSNAs in the mainstream smoke of cigarettes with functional reconstituted tobacco leaves decreased by 19.99% (Table 9).

TSNA reduction using complex cigarette filters

Some materials with hydroxyl bond have the ability of absorb TSNAs through formation of N…H-O bond, such as nanometer silica and macroporous silica gel, can be used to produce complex cigarette filters The TSNAs in the smoke of cigarettes with complex cigarette filter tip containing modified nanometer silica and macroporous silica gel could be reduced 24.11% compared with control cigarettes with cellulose acetate filter with the similar parameters (Table 10).

Application of the systematic TSNA-reducing technologies

Some of technologies for the reduction of TSNAs were gradually applied in the maintenance of cigarette products (i.e. ZNH-Jinzhuang 8mg). TSNA levels for the mainstream smoke of cigarette ZNH have been reduced gradually from 643ng/cig (2010) to 289ng/cig (2017) with a selective reduction rate of 53.7% with a relatively stable sensory quality. At the same time, the cigarette hazard index has also gradually decreased from 16.1 (2010) to 10.4 (2017), exhibiting a reduction ratio of 35.4% (Table 11).

All the above TSNA reduction technologies combined and applied in the development of cigarette (B). The application of the systematic TSNA reducing technologies could approach the selective reduction of 58% of TSNAs in cigarette smoke and 45% decrease of

Table 8: The levels of TSNAs in the mainstream smoke of cigarettes with cytochrome P450 recombinase processed tobacco and the control.

Sample	Tar/($mg.cig^{-1}$)	NNN/($ng.cig^{-1}$)	NAT/($ng.cig^{-1}$)	NAB/($ng.cig^{-1}$)	NNK/($ng.cig^{-1}$)	TSNAs/($ng.cig^{-1}$)
Control	7.10	185.44	84.72	8.03	24.34	302.53
Cigarettes with cytochrome P450 recombinase processed tobacco	7.5	182.1	81.6	8.0	17.3	288.9

Table 9: Effects of functional reconstituted tobacco leaves on TSNAs in cigarette smoke.

Sample	Tar/($mg.cig^{-1}$)	NNN/($ng.cig^{-1}$)	NAT/($ng.cig^{-1}$)	NAB/($ng.cig^{-1}$)	NNK/($ng.cig^{-1}$)	TSNAs/($ng.cig^{-1}$)
Control	6.1	146.2	63.4	6.3	17.4	233.3
Cigarettes with functional reconstituted tobacco	6.3	122.4	54.2	5.3	14.0	195.9

Table 10: Effect of complex cigarette filters containing modified nanometer silica and macroporous silica gel on TSNAs in cigarette smoke.

Sample	Tar/($mg.cig^{-1}$)	NNN/($ng.cig^{-1}$)	NAT/($ng.cig^{-1}$)	NAB/($ng.cig^{-1}$)	NNK/($ng.cig^{-1}$)	TSNAs/($ng.cig^{-1}$)
Control	7.23	198.77	89.68	9.11	25.02	322.58
Cigarettes with modified nanometer silicaand macroporous silica gel in the filter tip	7.4	155.0	70.7	7.1	20.0	252.9

Table 11: Level of TSNAs in the mainstream smoke of cigarette ZNH-Jinzhuang 8mg and cigarette hazard index for cigarette ZNH- Jinzhuang 8mg (2010-2017).

Year	Tar/($mg.cig^{-1}$)	NNN/($ng.cig^{-1}$)	NAT/($ng.cig^{-1}$)	NAB/($ng.cig^{-1}$)	NNK/($ng.cig^{-1}$)	TSNAs/($ng.cig^{-1}$)	H.Index
2010	7.6	420.3	170.2	12.8	39.4	642.7	16.1
2011	7.8	291.1	124.6	10.5	28.8	455.0	12.0
2012	8.3	230.4	100.6	10.1	23.5	364.4	10.9
2013	7.2	150.5	62.7	7.5	18.4	239.1	9.7
2014	7.6	211.0	95.6	9.0	22.4	338.0	10.7
2015	7.7	194.7	89.1	8.3	21.0	313.1	10.3
2016	7.5	180.5	80.5	8.0	20.1	289.1	10.5
2017	7.5	178.2	82.6	8.1	20.0	288.9	10.4

Table 12: TSNAs in cigarette smoke and cigarette hazard index for cigaretteB and the control.

Sample	Tar/(mg.cig^{-1})	NNN/(ng.cig^{-1})	NAT/(ng.cig^{-1})	NAB/(ng.cig^{-1})	NNK/(ng.cig^{-1})	TSNAs/(ng.cig^{-1})	H. Index
Control	7.3	190.1	86.3	8.8	24.7	309.9	13.5
B cigarette	7.2	75.0	35.7	4.4	9.6	124.7	7.4

Table 13: TSNA levelsfor the mainstream smoke of the cigarettes of ZNH brand and cigarette hazard index for ZNH-brand cigarettes (2010-2017).

Year	Tar/(mg.cig^{-1})	NNN/(ng.cig^{-1})	NAT/(ng.cig^{-1})	NAB/(ng.cig^{-1})	NNK/(ng.cig^{-1})	TSNAs/(ng.cig^{-1})	H. Index
2010	7.8	448.9	182.6	13.1	42.1	686.8	16.8
2011	7.7	161.2	67.8	7.9	17.2	254.1	10.2
2012	8.1	147.1	62.2	7.5	14.9	231.8	9.4
2013	7.6	143.2	60.8	7.5	13.5	226.3	9.2
2014	7.8	130.1	56.5	7.0	13.0	206.5	9.1
2015	7.8	140.5	61.3	7.5	13.5	222.9	9.0
2016	7.7	150.0	64.3	7.7	15.1	237.1	9.3
2017	7.7	132.6	58.4	7.1	14.8	212.9	9.2

cigarette hazard index, compared with the control (Table 12).

With the gradual application of a series of technologies for the reduction of TSNAs in the mainstream smoke of ZNH-brand cigarettes, TSNAs in the cigarette smoke were remarkably reduced from 686.79ng/cig (2010) to 212.86 ng/cig (2017) with a selective reduction ratio of 67.73%. At the same time, the cigarette hazard index was decreased from 16.8 (2010) to 9.2 (2017), exhibiting a reduction ratio of 45.24% (Table 13).

Conclusion

A series of innovative technologies have been systematically developed in agriculture and industry field, including tobacco breed improvement, cultivation, threshing and redrying, storage, processing and cigarette manufacture. The test results showed that all these nine technologies could effectively reduce the TSNA levels and provide practicable ways to reduce TSNAs in tobacco and cigarette smoke. The application of the combined TSNA reducing technologies could approach a selective reduction of 58% of TSNAs in cigarette smoke and the 45% decrease of cigarette hazard index.

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References

1. Shi H, Zhang J. Tobacco alkaloids. Beijing. China Agriculture Press. 2004.
2. Hecht SS. Biochemistry, biology, and carcinogenicity of tobacco-specificN-nitrosamines. Chem Res Toxico. 1998; 11: 559-603.
3. IARC. Evaluation of the carcinogenic risk of chemicals to humans: tobacco habits other than smoking: betel-quid and areca nut chewing and some related nitrosamines. France: International Agency for Research on Cancer Scientific Publications. 1985; 113-116.
4. Hecht SS, Hoffmann D. Tobacco-specific nitrosamines, an important group of carcinogens in tobacco and tobacco smoke. Carcinogenesis. 1988: 9: 875-884.
5. Burton HR, Dye NK, Bush LP. Relationship between tobacco-specific nitrosamines and nitrite from different air-cured tobacco varieties. J Agr Food Chem. 1994; 42: 2007-2011.
6. Bush LP, Cui M, Shi H, Burton HR. Formation of tobacco-specific nitrosamines in air-cured tobacco. Recent Advances in Tobacco Science. 2001; 27: 23-46.
7. Xie J. The principle and method of cigarette harm evaluation. Beijing: Chemical Industry Press. 2009.
8. Feng Q. Experimental analysis of reducing coke effect of filter ventilation dilution technolog. Tobacco Science &Technology. 2000; 7: 10-11.
9. Lian F, Li B, Huang C. The influence of filter nozzle ventilation on the combustion temperature of cigarette smoke and the seven harmful components in mainstream smoke. Hubei Agricultural Science. 2014; 53: 4074-4078.
10. Huang C, Li G, Lian F, Su M, Xie W, Zhang G, et al. The influence of cigarette paper characteristics on the release of 7 kinds of harmful components in mainstream cigarette smoke. Tobacco Science &Technology. 2011; 4: 29-32.
11. Dai L, Qiu B, Liang Y, Liu X, Li Q, Guo L, et al. The influence of filter nozzle dilution on the content of smoke aroma components in mainstream cigarette smoke was reviewed. Journal of Zhengzhou Light Industry Institute. 2014; 29: 33-38.
12. Cai J, Han B, Zhang X, Zhao X, Liu H, Xie F, et al. The influence of filter nozzle dilution on the release of some flavor components in mainstream cigarette smoke. Tobacco Science &Technology. 2011; 9: 54-60.
13. Zhou Y, Lin W, WanM, Yang J, Zhu J. Novel selective adsorbent derived from hierarchical rockery-like MCM-41 monolith. J MaterChem. 2012; 22: 23633-23641.
14. Meier WM, Siegmann K. Significant reduction of carcinogenic compounds in tobacco smoke by the use of zeolite catalysts. Micropor Mesopor Mat. 1999; 33: 307-310.
15. Lin W, Wan M, Zhou Y, Gu H, Zhou S, Zhu J. Novel selective catalyst derived from uniform clustered NaY zeolite microspheres. J Mater ChemA. 2013; 1: 6849-6857.
16. Zhao T, Shi H, Fan Z, Xu F, Wang X, Chu R, et al. Analysis of Alkaloid Contents and Nicotine Conversion Rate in Burley Tobacco from Binchuan. Tobacco Science &Technology. 2013; 6: 74-78.
17. Fan D, Liu X, Zhu B, Ye Ling, Zhe W, Xia J. Rapid determination of TSNAs in cut tobacco and mainstream cigarette smoke by UPLC-MS/MS. Acta Tabacaria Sinica. 2012; 18: 10-16.
18. Jing Y, Yang Y, Li G, Zhang H, Li H, Jin L, et al. Effects of Different Planting Densities on Neutral Aroma Components in Burley Tobacco. Acta Agriculturae Boreali-occidentalis Sinica. 2012; 21: 103-107.
19. Zhang J, Bai R, Yi X, Yang Z, Liu X, Zhou J, et al. Fully automated analysis of four tobacco-specific N-nitrosamines in mainstream cigarette smoke using two-dimensional online solid phase extraction combined with liquid

chromatography-tandem mass spectrometry. Talanta. 2016; 146: 216-224.

20. Wang J, Yang H, Shi H, Zhou J, Bai R, Zhang M, et al. Nitrate and nitrite promote formation of tobacco-specific nitrosamines via nitrogen oxides intermediates during postcured storage under warm temperature. Journal of Chemistry. 2017; 2017: 1-11.

21. Shi H, Wang R, Bush LP, Zhou J, Yang H, Fannin N, et al. Changes in TSNA contents during tobacco storage and the effect of temperature and nitrate level on TSNA formation. Agric Food Chem. 2013; 61: 11588-11594.

22. Wang J, Yang H, Shi H, Jin T, Zhou J, Bai R. Interacting effects of temperature moisture content and storage environment on tobacco-specific nitrosamines formation during burley tobacco storage. Fresen Environ Bull. 2017; 3: 2112-2123.

23. Liu X, Zhang J, Zhang C, Yang B, Wang L, Zhou J. The inhibition of cytochrome P450 2A13-catalyzed NNK metabolism by NAT-NAB and nicotine. Toxicology Research. 2016; 5: 1115-1121.

24. Liu X, Zhang J, Wang L, Yang B, Zhang C, Liu W, et al. *In vitro* metabolism of N-Nitrosonornicotine catalyzed by cytochrome P450 2A13 and its inhibition by nicotine N-Nitrosoanatabine and N-nitrosoanabasine. Chemico-Biological Interactions. 2016; 260: 263-269.